

THE DEVELOPMENT OF A NEW TRACHEAL STENOSIS MODEL
IN PIGLETS AND ITS REPAIR WITH A
FREE PERIOSTEAL GRAFT

by

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DECLARATION

I declare that I, Ralph Clinton Cohen, am the sole author of this dissertation and that all references cited have been consulted personally. The work, of which this thesis is a record, has been performed by me and has not been previously accepted for a higher degree.

Signed Ralph Cohen
 Date 23.5.88.

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7 Three examiners shall be appointed to examine the thesis, not less than two being external.

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ABSTRACT

Airway narrowing in children from congenital stenosis or acquired stricture remains a difficult and challenging surgical problem. When the segment of tracheal stenosis is short, the best surgical treatment is to excise the stenosis and perform a primary end-to-end anastomosis. When there is an extensive segment of tracheal stenosis, this is clearly not possible and another means of enlarging the airway has to be devised. An appealing technique is to incise the stenotic segment longitudinally throughout its length, insert an endotracheal stent and then repair the resultant defect in the trachea with a suitable graft material.

In this series of experiments, this technique is examined in the thoracic segment of the piglet trachea. The study is performed in two phases. The initial experiments involve the normal trachea in which a window defect is created over the entire length of the thoracic trachea involving 30% of its circumference. This defect represents that which would result when a stenotic segment of trachea is incised longitudinally. The window defect is then repaired with a free tibial periosteal graft. In the second phase, a model of tracheal stenosis is created by encircling the thoracic trachea with a silastic sheet. Once respiratory distress is established, the silastic sheet is removed at a second operation, and the stenotic segment incised,

an endotracheal silastic stent inserted through the defect and the defect repaired with free tibial periosteal graft. The pigs are sacrificed three months later when they have grown to about six times their original weight and the trachea removed. These studies demonstrate that free tibial periosteal grafts are an ideal biological material for repairing tracheal defects. The periosteum forms bone in a short period of time and supports the airway; the luminal surface is lined with columnar and respiratory epithelium.

This model suggests that tracheal stenosis in children may be surgically corrected by simply incising the stenotic segment longitudinally and enlarging the tracheal diameter by inserting a free periosteal graft into the defect.

INTRODUCTION

Airway narrowing from extensive congenital stenosis or acquired stricture is commonly a lethal condition for which there is currently no satisfactory surgical solution.

It is difficult to determine the true incidence of tracheal stenosis from the sporadic case reports and the paucity of large retrospective reviews from individual Paediatric centres. A recent review (Benjamin, 1981) of congenital tracheal stenosis, during the 10-year period 1971 to 1980, revealed 21 cases at the Royal Alexandra Hospital for Children, Sydney, a major Paediatric hospital with 7,000 surgical admissions a year. In this review, one third of patients with congenital airway narrowing died before seven months of age, due to respiratory failure. The onset of symptoms depends on the extent and severity of the airway narrowing. Some children present early in the first month of life whilst others may remain relatively asymptomatic until seven years of age (Cantrell, 1964). Airway obstruction is commonly precipitated by a viral respiratory tract infection and presentation is often dramatic (Fonkalsrud, 1971; Kimura, 1982; Benjamin, 1982; Idriss, 1984). Attempts at endotracheal intubation may not be successful because of the fixed narrow calibre of the trachea and death is due to acute airway obstruction.

Associated anomalies of the respiratory tract

and oesophagus are not uncommon; aplasia of a lung, H-type tracheo-oesophageal fistula and aberrant left pulmonary artery have all been reported (Benjamin, (1981). However, none of these co-existing anomalies are lethal or incapable of correction.

Pathology

There are three morphological variants of congenital tracheal stenosis (Cantrell, 1964).

1. Generalized hypoplasia. The entire trachea is narrow in calibre with posterior fusion of the individual cartilagenous rings. The diameter of the lumen may vary from 1 to 3 mm in the newborn.
2. Funnel-like stenosis. The tracheal diameter becomes progressively narrower to the level of the bifurcation. This is the least common variant.
3. Segmental stenosis. A portion of the trachea is narrowed in an hour glass fashion. This is the most common form of the anomaly. The length of the stenotic segment varies from 1 to 5cm and may be found in any portion of the trachea.

Histologic evaluation demonstrates cartilagenous rings which are abnormal in shape and fused posteriorly with mild chronic inflammatory changes in the submucosa and an increased amount of dense collagen in the wall of the trachea (Cantrell 1964).

Evolution of Current Treatment Concepts

The earliest experiments in tracheal reconstruction are credited to Gluck and Zeller who performed a tracheal transection and re-anastomosis of the dog trachea in 1881 (Bailey, 1969, 1970). In 1911, Hohmeyer successfully repaired window defects in dogs' tracheas with fascia lata (Bucher, 1951).

In the late nineteenth and early twentieth centuries, multiple techniques were developed to cover defects created by partial circumferential resection of the trachea. These consisted of skin flaps, free skin grafts, or fascia that were often stiffened with needles, silver gauze, cartilage or bone (Konig, 1896; Fergusson, 1950). These methods met with varying degrees of success.

Currently, surgical techniques used to correct extensive tracheal stenosis include:

1. Resection and anastomosis.
2. Resection and insertion of a prosthesis and
3. Incision and grafting.

It has generally been accepted that resection and end-to-end anastomosis is the ideal treatment for tracheal stenosis (Grillo, 1970, 1972). However, this is often not possible in congenital tracheal stenosis because of the extent of the trachea involved. In 1972, Neville reported the first successful replacement of the intrathoracic trachea, carina and mainstem bronchi with a molded

bifurcated silicone prosthesis in a human (Neville, 1972). Generally, prosthetic replacement of the trachea is not a suitable alternative as the foreign material usually becomes infected and granulation tissue forms, causing obstruction to the airway (Bailey, 1970). The use of prostheses would certainly lead to problems in young growing individuals. The final alternative, incision and grafting is applicable to most cases. This involves longitudinal incision of the stenotic trachea and insertion of a graft into the defect thereby enlarging the tracheal diameter. A multitude of tissues have been used for this purpose including fascia, skin, bone, periosteum, cartilage, perichondrium, tracheal allograft, oesophagus and pericardium (Belsey, 1950; Fonkalsrud, 1966; Fonkalsrud, 1971; Olsen, 1976; Kufaas 1981, Kimura, 1982; Idriss, 1984).

Free periosteal grafts have been used experimentally with variable success to repair window defects in the cervical trachea of rabbits (Fonkalsrud, 1966; Ritsila, 1973). The viability of these free periosteal grafts was directly related to the blood supply. In the cervical trachea, the free periosteal grafts can readily obtain a blood supply from the overlying strap muscles (Kufaas 1981). However, the majority of congenital tracheal stenoses in humans occur in the thoracic trachea where vascularized muscle is not

readily available to apply to the free periosteal graft. In an attempt to overcome this problem, viable pedicle grafts of costal periosteum have been used successfully in dogs to repair the thoracic tracheal defects (Penton, 1951; Blair, 1958; Fonkalsrud, 1966). However, the length of the vascular pedicle severely restricts the length of the tracheal defect which can be repaired. In a recent study (Morgan, 1982) the problem of providing a blood supply to a bronchial allograft was successfully overcome by applying an omental pedicle graft. This technique is utilized in the present study to assess the need for additional vascularization of the free tibial periosteal graft in the thoracic tracheal defects.

All previous experimental models have created defects in the normal trachea to assess various methods of repair. This has cast serious doubts on the potential application of these repairs to the pathological trachea of congenital tracheal stenosis seen in humans.

The following experiments describe the development of a simple reproducible technique for creating a model of tracheal stenosis which clinically and histopathologically resembles congenital tracheal stenosis seen in children and its successful repair using free tibial periosteal grafts.

Objects of the Investigation

The first phase is to devise a technique for enlarging the tracheal diameter over an extensive segment of thoracic tracheal stenosis.

Initially, the suitability of tibial free periosteal graft will be assessed for repair of a defect in the normal thoracic trachea of piglets. This defect represents that produced when the thoracic tracheal stenosis is treated by longitudinal incision.

The second phase of the experiment aims at creating a suitable model of tracheal stenosis in the thoracic trachea which more closely resembles the clinical problem of congenital tracheal stenosis seen in children and then to incise this longitudinally and insert a free tibial periosteal graft. In addition, an omental pedicle graft will be applied to the free periosteal grafts in some animals to determine if this is necessary to ensure vascularization of the free periosteal graft.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by National Institutes of Health (NIH Publication No. 80-23, revised 1978).

MATERIALS AND METHODS

Phase 1: Thoracic Tracheal Window Defect and its Repair

Pre-operative Preparation

Piglets (9.9 ± 1.0 kg) were used because the tracheal size is similar to that of a young child and they grow rapidly, increasing their weight 4 to 6 fold over a 3 month period. Each of nine piglets was premedicated with atropine sulphate (0.03mg/kg) and Cefazolin (50mg/kg) was given intramuscularly, one hour prior to surgery. The animal was anaesthetized with nitrous oxide, oxygen, and halothane, shaved appropriately and scrubbed with chlorhexidine solution. A size 5 cuffed endotracheal tube was inserted and ventilation maintained with a ventilator. An intravenous cannula was inserted in an ear vein and 5% dextrose with 0.2% normal saline was infused (10ml/kg/hr). Just prior to surgery the operative fields were washed with 70% alcohol and 10% povidone iodine.

Operative technique

The piglets were placed in a right lateral position and sterile drapes placed appropriately. A right lateral thoracotomy incision was made and the pleura entered through the third intercostal space, the third costosternal junction was divided. The lung was retracted inferiorly to expose the

trachea above the right upper lobe bronchus and the azygos vein was ligated with 2/0 silk and divided. The pleura was incised and reflected anteromedially from the surface of the trachea along with the vagus nerve. The external circumference and diameter of the trachea were measured.

A 3 to 3.5cm long (7-10 rings) and 1 to 1.5cm wide rectangular segment was removed from the right lateral tracheal wall from the thoracic inlet to 1cm above the right upper lobe bronchus. About 30% of the circumference of the entire thoracic trachea was excised. A Silastic/Marlex internal splint (Fig.1) was inserted through the defect to seal the airway. The splint fitted snugly in the trachea and extended beyond the upper and lower limits of the defect (Fig.2). A single 4/0 Vicryl suture was used to secure the splint to the inferior margin of the defect to prevent movement.

A vertical incision was made over the left tibia and an appropriately sized rectangular flap of tibial periosteum was excised. The graft was sutured to the margins of the defect using interrupted 5/0 Vicryl sutures with the osteogenic layer facing toward the tracheal lumen. Adjacent pleura was sutured over the graft with 5/0 Vicryl. Normal saline was instilled into the chest, the right lung was re-expanded, and any air leaks from the graft site were controlled with additional Vicryl sutures.

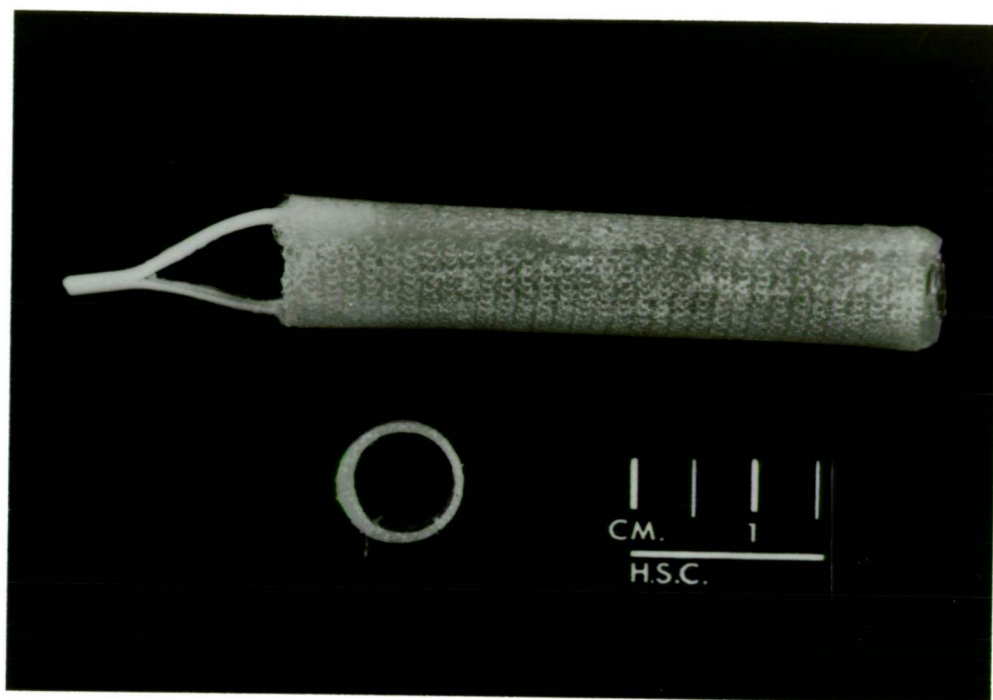


Fig. 1 Silastic/Marlex endotracheal splint. Handle used for endoscopic removal.

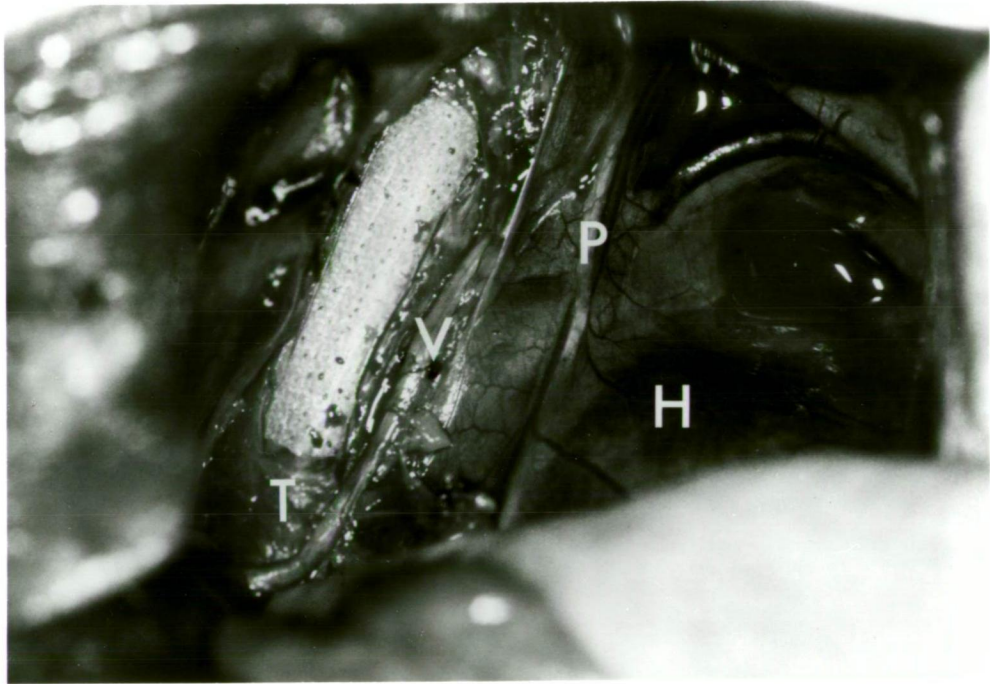


Fig. 2. Tracheal defect with endotracheal splint in Place.

H-Heart P-Phrenic nerve: V-Vagus nerve:
T-Trachea.

In four piglets, a vertical midline upper abdominal incision was made to mobilize an adequate length of greater omentum. This omental pedicle graft (OPG) was then rotated upwards into the chest via the retrosternal route and sutured to the surface of the FPG with 5/0 Vicryl.

A size 16 chest drain was inserted through a separate stab incision, the chest closed, the lungs inflated, and the chest drain removed. All the skin incisions were closed with continuous 3/0 Prolene suture.

Post-operative Management

All piglets were given a single dose of Cefazolin 50mg/kg intramuscularly for seven days.

Two weeks post-operatively the piglets were anaesthetized, weighed, and the endotracheal splint was removed using a flexible fiberoptic bronchoscope and flexible alligator forceps. A lateral chest X-ray was taken.

Assessment of tracheal window defect repairs

The animals were killed three months post-operatively. The trachea was removed, measured, and then radiographed using a Faxitron 43805 X-ray System (Hewlett-Packard Co., Palo Alto, Calif.) with high resolution, Kodak X-omat TL film at 35 KvP for 35msec.

Transverse sections were taken from normal

trachea 1 to 2cm above the repair site and at the mid-repair level. These were fixed and stained with haematoxylin-phloxine-saffron and studied by light microscopy. Sections were also fixed and examined by scanning electron microscopy.

External circumference and internal area measurements were made from the histopathology slides using the IBAS image analyzer (Carl Zeiss, Inc. Thornwood, NY) at sites in the normal and in the grafted trachea. The MINITAB computer statistical package was used to calculate a linear regression equation relating the normal external tracheal circumference to the internal cross-sectional area: (Fig. 3)

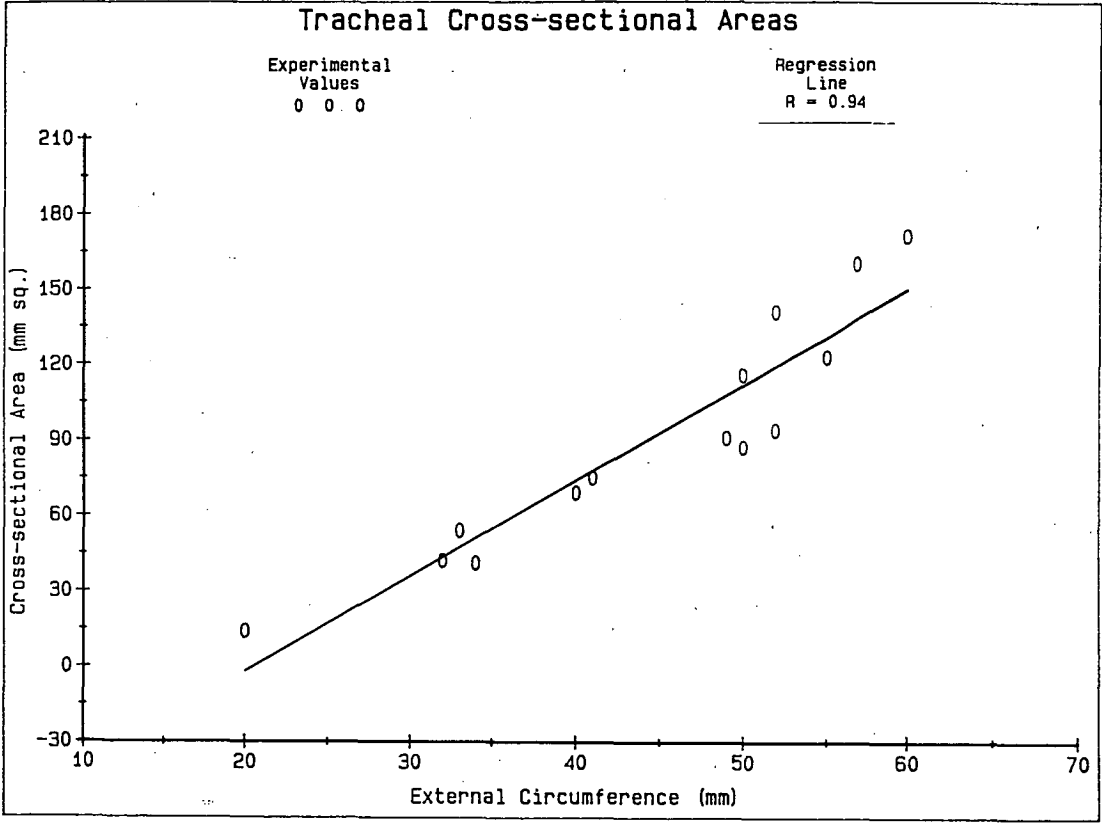
$$\text{Area (mm}^2\text{)} = -77.8 + 3.79 \times \text{Circ (mm):}$$

$$(R = 0.94). \quad (\text{Cohen, 1985})$$

Since the initial cross-sectional area of the trachea could not be measured in vivo, the tracheal circumference was measured and this equation then used to predict the internal cross-sectional area of the trachea at the time of repair.

A measurement of normal tracheal growth over the 3-month period was then obtained by comparing initial tracheal cross-sectional areas with those measured from the histopathology slides in the normal trachea above the repair. A further comparison was made between the cross-sectional area above the repair and at mid-repair to determine the effect of the graft on tracheal

Fig.3 Graph of linear regression equation relating normal external pig trachea circumference to internal cross sectional area.



growth after 3 months. A paired t-test was performed to assess significant differences. Also, the width of the graft was compared to the overall tracheal circumference at mid-repair to determine the relative growth rates of the graft and normal tracheal ring.

In one pig, both tibiae were removed: radiologic and histopathologic comparisons were made to detect any adverse effects from removing the periosteum.

Phase II: Tracheal Stenosis Model and its Repair

Tracheal stenosis was produced in 17 piglets by encircling the thoracic trachea in 15 piglets (7.7 ± 1.1 kg) and the cervical trachea in 2 piglets (2kg) with an appropriate sized piece of Silastic sheeting.

When severe respiratory obstruction developed from 3 to 19 days later (median 14 days), all animals were re-operated on and the Silastic cuff removed. In four controls, no further treatment was given. When it was established that respiratory obstruction was not relieved, the pigs were put to death.

In 13 piglets, operative repair of the thoracic tracheal stenosis was undertaken immediately following removal of the cuff.

Operative Techniques

All animals were premedicated with 0.3mg atropine sulphate intramuscularly and anaesthetized with nitrous oxide, oxygen and halothane through an endotracheal tube or an anaesthetic mask when cervical tracheal stenosis was created. The skin was shaved and scrubbed with chlorhexidine soap; 70% alcohol and 10% povidone iodine was applied just prior to incision.

All piglets were given Cefazolin (70mg/kg) intramuscularly, one hour pre-operatively and for seven days after each operation.

To create the cervical tracheal stenosis, the entire cervical trachea was exposed by a mid-line incision retracting the strap muscles laterally and the thyroid gland inferiorly. The circumference and diameter of the trachea was measured. The trachea was encircled with Silastic sheet, 1mm thick and 2cm long (9 tracheal rings). The sheet circumference was 3mm greater than the tracheal circumference so that it did not constrict the trachea.

The free margins of the Silastic sheet were sutured to each other with four 4/0 Prolene sutures which also included a superficial "bite" of the underlying trachea to prevent migration of the cuff. The wound was closed in layers with 4/0 Vicryl and continuous 3/0 Prolene sutures to the skin.

To create the thoracic tracheal stenosis, the entire thoracic trachea proximal to the right upper lobe bronchus was exposed via a right lateral extrapleural thoracotomy through the third interspace. This gave complete access to the entire thoracic trachea proximal to the right upper lobe bronchus. The tracheal diameter and circumference were measured and a Silastic sheet was applied to the circumference of the trachea as described above (Fig. 4A,B).

Pericostal sutures of 2/0 Vicryl were used to approximate the ribs. The muscles were closed in layers with 3/0 Vicryl and 3/0 Prolene sutures were used for skin. A chest drain was not used.

When the second operation became necessary because of airway obstruction, the previous thoracotomy incision was opened. The pleura was entered and the lung retracted inferiorly to reveal the pseudocapsule surrounding the Silastic cuff. This was incised longitudinally and the cuff was removed.

To repair the stenosis, the trachea was incised longitudinally in its right antero-lateral wall over the stenotic segment. A Silastic endotracheal splint was inserted through the tracheal defect to seal the airway (Fig. 5A,B). The external diameter of this tube was 9.5mm., the same as the internal diameter of the normal trachea above the stenosis. The splint was sutured in



Fig 4A. Silastic sheet used to encircle the trachea.

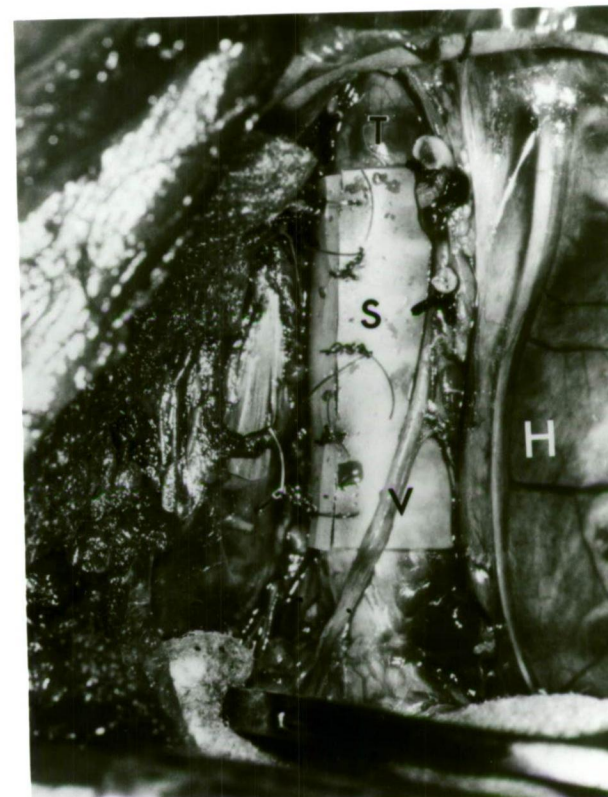


Fig 4B. Silastic cuff (S) encircling the thoracic trachea (T)
H-Heart: V-Vagus Nerve.

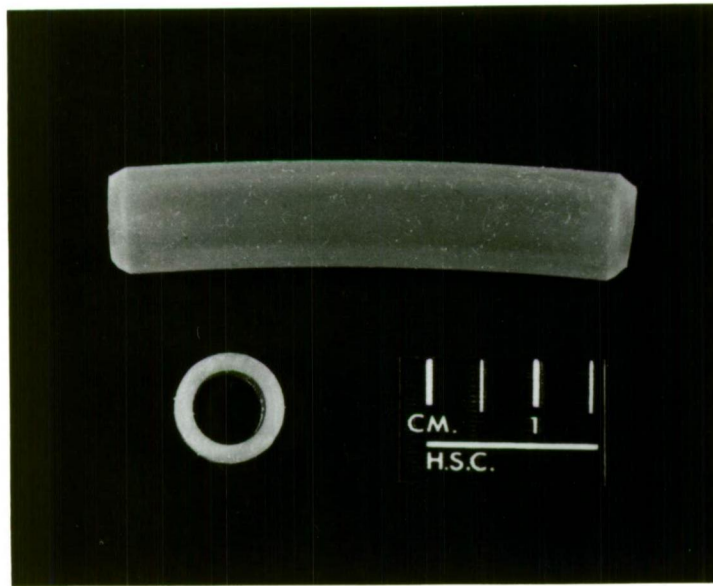


Fig 5A. Endotracheal Silastic splint.

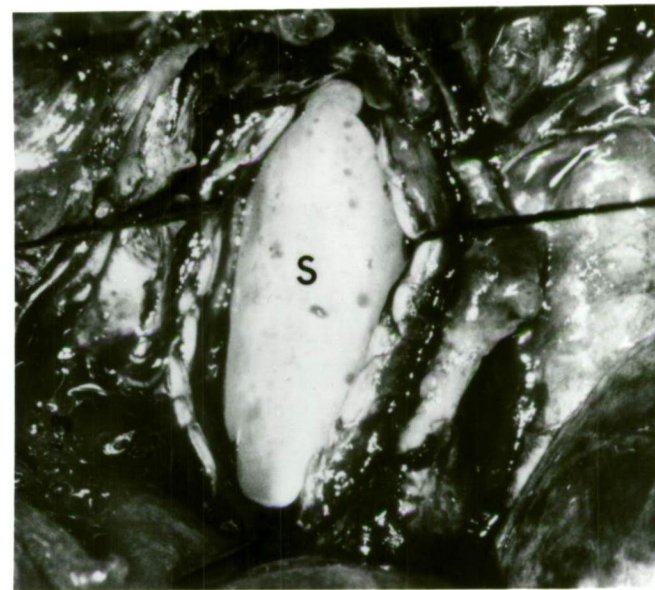


Fig 5B. Stenotic trachea incised longitudinally
and Silastic splint inserted

place with a 3/0 Vicryl suture so that its lower end did not occlude the origin of the right upper lobe bronchus. A vertical incision was made over the left tibia and a 3cm x 1.5cm wide rectangular flap of tibial periosteum was excised. The periosteum with the osteogenic layer facing inwards, was sutured to the margins of the tracheal defect with interrupted 5/0 Vicryl.

The chest was closed as already described. In three of these piglets, a vertical midline upper abdominal incision was made to mobilize an omental pedicle graft (OPG) from the greater curve of the stomach. The OPG was rotated upwards into the chest via the retrosternal route and sutured to the surface of the FPG with 5/0 Vicryl sutures.

A size 16 chest tube was inserted through a separate stab incision and after the chest was closed and the lungs inflated, the chest tube was removed.

Three weeks after the repair, the piglets were anaesthetized with 75 to 150mg of sodium pentobarbital intravenously to remove the endotracheal splint. Under X-ray image intensifier control, the splint was grasped and removed with a peanut forceps which had been passed down the lumen of a 4.5 endotracheal tube. The trachea was then examined with a flexible fiberoptic bronchoscope and a lateral chest X-ray was obtained.

Assessment of trachea following stenosis

Lateral cervical or chest radiographs were

taken when respiratory obstruction became evident clinically. Tracheal stenosis was calculated by comparing the internal sagittal diameter of the normal trachea to the diameter at mid-stenosis.

To confirm that the radiographic assessment of tracheal stenosis was accurate, radiographic measurements were compared to actual measurements made in the four control (untreated) animals at sacrifice. Transverse sections of the tracheas above the stenosis and at mid-stenosis were fixed, stained and mounted on histopathology slides and the internal cross-sectional areas measured using the IBAS image analyzer (Carl Zeiss).

Animals that underwent repair of the tracheal stenosis were sacrificed 1 month (n=1), 2 months (n=1) and 3 months (n=10) after repair. The trachea was removed and radiographs were obtained using a Faxitron 43805 X-ray System (Hewlett-Packard) with high resolution Kodak X-Omat TL film at 35kvp for 35 msec.

Transverse sections taken from the normal trachea 1 to 2cm above the repair site and at mid-repair level were fixed and stained with haematoxylin-phloxine-saffron and studied by light microscopy. Cross-sectional area measurements were made from the histopathological slides using the IBAS image analyzer (Carl Zeiss, Inc., Thornwood N.Y). Sections were also examined by scanning electron microscopy.

To determine the internal cross-sectional area of the stenotic trachea at the time of repair, indirect measurements were necessary according to the equation:

$$\begin{aligned} &\text{Cross-sectional area of stenotic trachea} = \\ &\text{Cross-sectional area of normal trachea} \times \% \\ &\text{stenosis (from X-ray).} \end{aligned}$$

Radiographic measurements of the airway were used to determine the degree of stenosis (expressed as a percentage). The cross-sectional area of the normal trachea was determined from a linear regression equation previously shown to relate external tracheal circumference of the normal pig trachea to its internal cross-sectional area (Cohen, 1985).

Tracheal growth three months after repair was determined by comparing the cross-sectional area of the trachea prior to creation of stenosis with the cross-sectional areas of trachea above the repair and at mid-repair. A paired t test was performed to assess significant differences.

RESULTS

Thoracic Tracheal window defect and its repair

The piglets were ambulatory and eating within 24 hours of operation. None of the animals developed signs of airway obstruction at any stage following the repair. One pig died unexpectedly on day 18 postoperatively; the cause could not be determined. Pig number four started vomiting on the 13th day after repair. At laparotomy 60cm of small bowel had infarcted due to intussusception. This was resected and the pig survived but its weight gain was less than the others. The mean weight of the piglets increased from 9.9 ± 1.0 kg at operation to 50 ± 13 kg at death 90 ± 7 days later.

The mean external circumference of the normal trachea increased from 36.7 ± 2.6 mm at operation to 53.1 ± 3.9 mm at death. There was no limitation of growth evident on the external surface of the tracheas. The mean cross-sectional area of the normal trachea doubled over the 3-month period. The internal cross-sectional areas at the mid-repair site after 3 months were the same or significantly greater than the normal trachea above the repair (Table 1). The grafts retained their original dimensions and the increased size of the trachea was due to growth of the normal tracheal rings. All grafts had ossified and histologic sections showed an outer ring of cortical bone surrounding central marrow spaces with

Table 1 Summary of Tracheal Cross-Sectional Areas at Operation and Sacrifice

Pig	Days to death	Weight at death (kg)	Cross-sectional areas (mm ²)			*Graft
			at operation **(predicted)	at sacrifice Above repair (control)	Mid-repair	
1	95	54	59.0	140.6 \pm 0.9	138.5 \pm 2.6	FPG/OPG.
2	96	60	47.0	116.1 \pm 1.7	*132.1 \pm 1.2	FPG/OPG.
3	77	41	74.0	93.5 \pm 0.9	*112.5 \pm 1.0	FPG/OPG.
4	87	27	55.0	87.2 \pm 1.4	*105.3 \pm 0.9	FPG/OPG.
5	94	66	59.0	170.9 \pm 0.8	*183.0 \pm 0.4	FPG
6	95	61	55.0	159.6 \pm 1.4	160.7 \pm 1.1	FPG
7	86	41	74.0	123.4 \pm 1.2	*137.6 \pm 1.4	FPG
8	90	48	55.0	90.5 \pm 0.8	*112.8 \pm 0.7	FPG
9 [†]	18	11	74.0	75.4 \pm 0.3	* 70.6 \pm 0.2	FPG.

**From linear regression equation

*p < 0.05

[†]Died unexpectedly

*All grafts ossified and epithelialized

FPG = Free periosteal graft.

OPG = Omental pedicle graft.

hematopoietic elements. Beneath the interposed osseous tissue and the lumen was a dense layer of well-collagenized fibrous tissue covered with ciliated columnar epithelium (Fig. 6). The cilia were also well shown on scanning electron microscopy (Fig. 7A,B).

In four repairs, an unusual feature of epiphyseal plate induction was seen at the ends of the tracheal cartilage rings where they adjoined the ossified periosteum (Fig. 8).

Ossification and epithelialization of the FPG was not improved by application of omental pedicle graft.

At 18 days post-operatively, squamous epithelium was seen in the central part of the graft with columnar ciliated epithelium at the edges. The more simple epithelium transformed to columnar ciliated cells typical of the respiratory tract, commencing at the margins of the graft and progressing toward the centre.

The trachea above the grafted area demonstrated normal respiratory epithelium without evidence of inflammation. Regular examination of the hind legs in all animals did not demonstrate any discrepancy in growth between the operated and non-operated tibiae. Histologic and radiographic examination of both tibiae in one animal confirmed this impression.

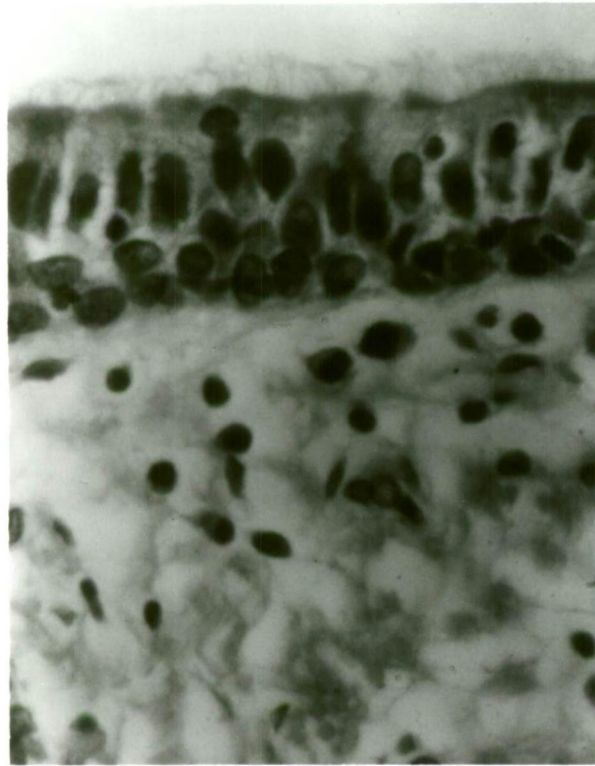


Fig 6A. Normal tracheal epithelium

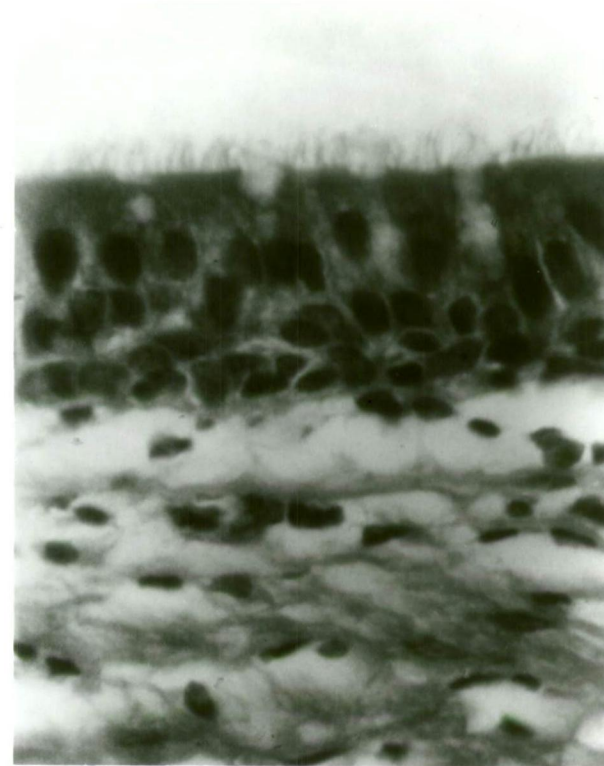


Fig 6B. Regenerated tracheal epithelium
over the graft surface

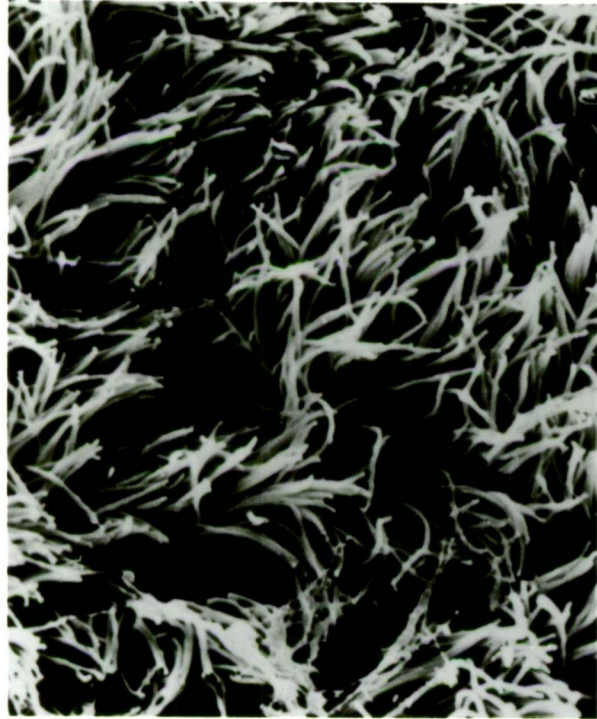


Fig 7A. Scanning electron micrograph of normal tracheal epithelium (x 12,600)

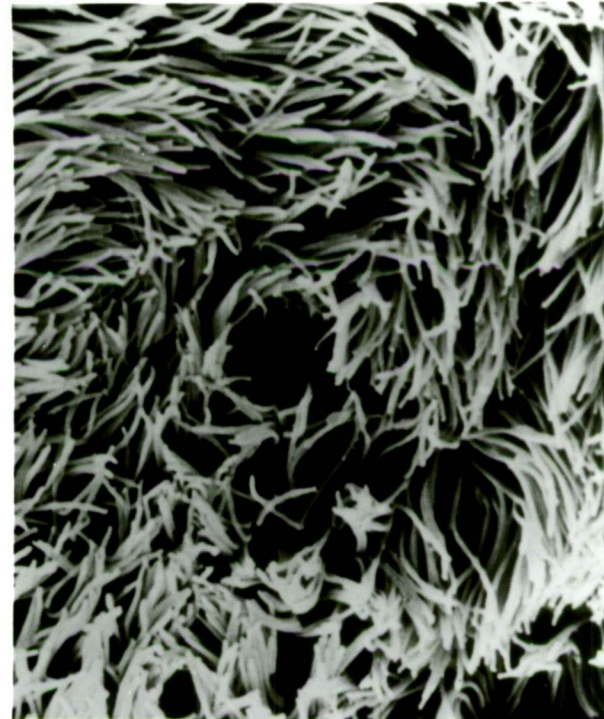


Fig 7B. Epithelium over the luminal surface of the graft (x 12,600)

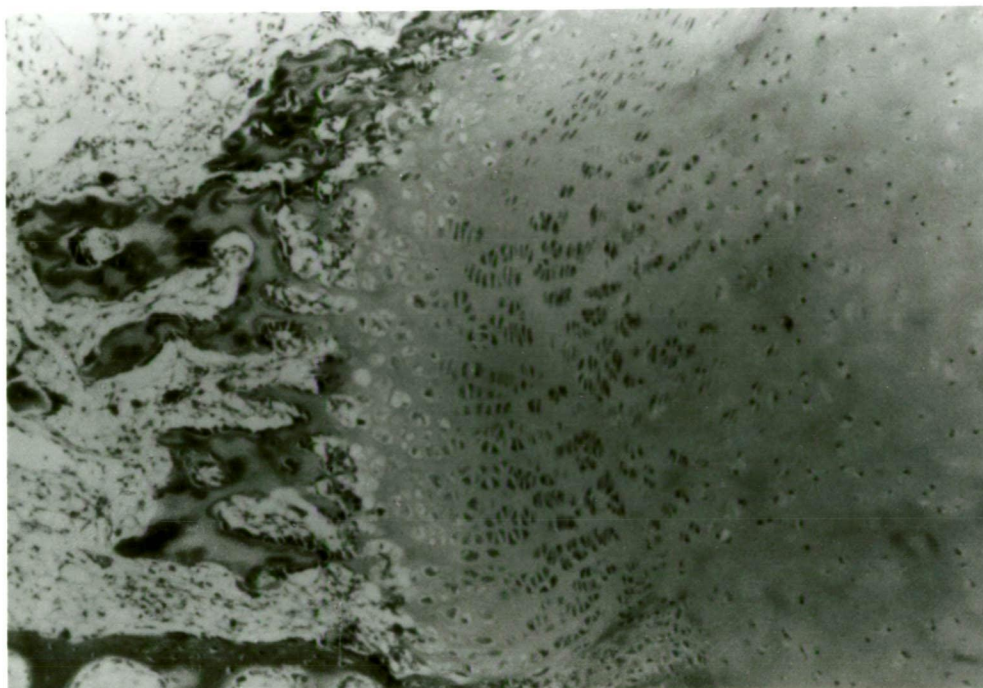


Fig 8. Epiphyseal plate induction at the graft/cartilage junction

Tracheal Stenosis model and its repair

The Stenosis model

The Silastic cuff caused overlap and infolding of the tracheal rings along the posterior aspect of the trachea. Tracheal stenosis became permanent when this invaginated membranous trachea became surrounded by fibrous tissue (Fig. 9).

Airway obstruction became apparant a median of 14 days following application of the Silastic cuff. Radiographic assessment demonstrated 60 to 70% reduction in the airway diameter in the stenotic segment (Fig. 10). Removal of the cuff in four animals did not relieve airway obstructin and these piglets were put to death. The percentage airway reduction recorded by actual measurement of tracheal cross-sectional areas above the stenosis corresponded satisfactorily with that determined by radiographic assessment (Table 2).

The Repair model

The pigs were ambulatory and eating within 24 hours of repairing the stenosis. There were no signs of airway obstruction at any stage following repair in 12 pigs.

Problems with the repair were noted in two of the thirteen animals. One pig developed a persistent cough three weeks following surgery and died a week later. Post mortem examination

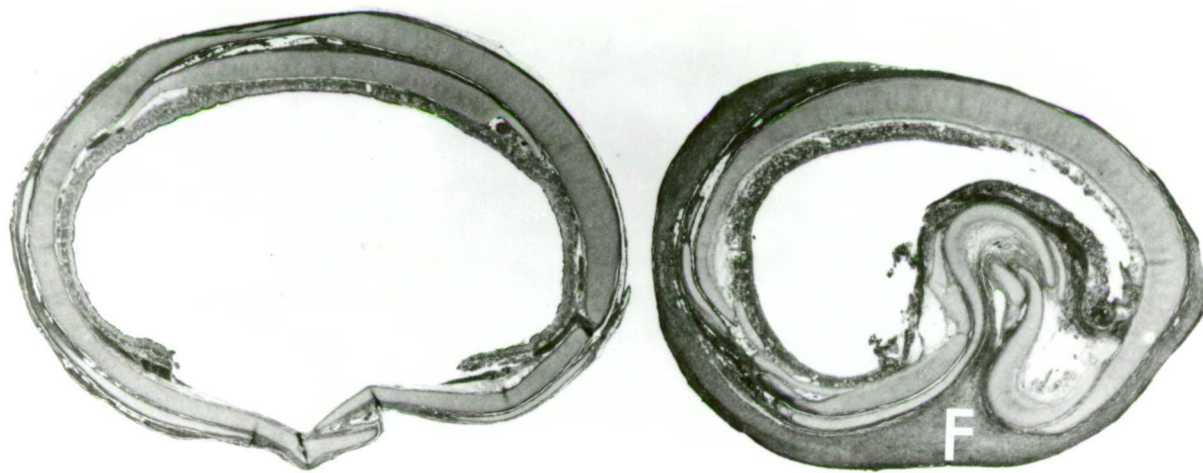


Fig 9. Normal trachea above stenosis (left). Permanent stenosis created by invagination of membranous trachea (right). Fibrous tissue (F).



Fig 10. Post-mortem radiograph of trachea 14 days after application of Silastic cuff (70% stenosis).

Table 2 Comparison of radiographic and cross-sectional area measurements of tracheal stenosis in control pigs

<i>Pig</i>	<i>Days to death</i>	<i>Weight at death (kg)</i>	<i>Cross-sectional areas* (mm²)</i>		<i>Percent stenosis measured</i>	<i>Percent stenosis from x-ray films</i>
			<i>Above stenosis</i>	<i>Mid-stenosis</i>		
1 (thoracic)	15	7	39.8 ± 0.3	16.5 ± 0.8	60	70
2 (thoracic)	14	7	48.8 ± 0.9	14.6 ± 0.7	70	80
3 (cervical)	13	2	13.5 ± 0.3	4.7 ± 0.1	65	70
4 (cervical)	13	2	18.8 ± 0.1	5.6 ± 0.1	70	70

*Measured from transverse sections by IBAS image analyzer.

revealed a staphylococcus aureus chest wound infection and concentric narrowing of the trachea to 70% of the lumen due to granulation tissue at the lower end of the graft, extending over a 3mm length. In another pig, an unsuspected short segment of concentric stenosis (24% of the normal tracheal cross-sectional area over a 2mm length) was noted when the animal was put to death three months after repair.

In both animals, the grafts had not calcified but were entirely replaced with fibrous tissue. The cut ends of the cartilagenous rings were in close proximity and contributed to the stenosis. However, in both, the cross-sectional areas of the repaired segments above and below the stenotic area were greater than the normal trachea above the area of repair.

Mean bodyweight increased from $7.7 \pm 1.1\text{kg}$ at the time of original operation to $56.0 \pm 9.3\text{kg}$ when the animals were put to death 90 ± 2 days later. The non-operated tracheal cross-sectional area more than doubled from an initial mean of $49 \pm 10\text{ mm}^2$ to $114.8 \pm 11.5\text{ mm}^2$ three months later.

At autopsy, the tracheal internal cross-sectional areas at the mid-repair site were significantly greater than the normal trachea above the repair and this was evident as early as 22 days post-operatively (Table 3).

The mean internal cross-sectional area of the

Table 3 Tracheal cross-sectional areas at stenosis and when animal was put to death

Pig	Days to death	Percent stenosis from x-ray films	Cross-sectional areas (mm ²)				Ossification of graft
			Stenosis		Death		
			Above stenosis*	Mid-stenosis†	Above repair (control)	Mid-repair	
1	26	70	66	20	71.3 ± 0.6	22.5 ± 0.2	Nil (FPG)
2	90	80	55	11	112.8 ± 1.6	86.3 ± 0.5	Nil (FPG)
3	90	70	62	19	101.7 ± 0.5	104.2 ± 0.9‡	Partial (FPG)
4	90	70	40	12	116.8 ± 0.9	136.1 ± 0.7‡	Partial (FPG)
5	64	80	32	6	76.1 ± 0.7	139.8 ± 0.7‡	Partial (FPG)
6	22	50	32	16	53.8 ± 0.7	73.6 ± 0.3‡	Complete (FPG)
7	90	80	55	11	132.1 ± 0.4	170.3 ± 0.6‡	Complete (FPG)
8	87	70	40	12	129.1 ± 1.2	184.8 ± 0.7‡	Complete (FPG)
9	88	70	36	11	118.6 ± 1.0	189.8 ± 0.6‡	Complete (FPG)
10	92	60	55	22	120.4 ± 0.6	191.5 ± 0.8‡	Complete (FPG)
11	82	70	55	17	94.3 ± 0.5	114.4 ± 1.2‡	Complete (FPG/OPG)
12	90	70	55	17	107.6 ± 1.3	150.7 ± 0.9‡	Complete (FPG/OPG)
13	90	70	55	17	114.8 ± 1.2	147.2 ± 0.3‡	Complete (FPG/OPG)

Legend: FPG, Free periosteal graft. OPG, Omental pedicle graft.

*Derived from measurement of circumference and linear regression equation.

†Derived from radiographic measurement of percent stenosis and calculated cross-sectional area of trachea above stenosis.

‡p < 0.05.

stenosed trachea increased approximately tenfold from $14.1 \pm 4.5\text{mm}^2$ to $147.5 \pm 37.3\text{mm}^2$ three months following repair. The grafts retained their original dimensions and the increased size of the trachea was due to growth of the tracheal rings.

Histologic review demonstrated ossification in 11 of the grafts. In 8, there was complete ossification (Fig. 11) and in 3, ossification was patchy and incomplete. An outer ring of cortical bone surrounded central marrow spaces with haematopoietic elements.

Beneath the interposed osseous tissue and the lumen was a layer of well organized fibrous tissue as thin as 0.04mm in places. Patches of ciliated epithelium were demonstrated on scanning electron microscopy 22 and 64 days post repair. After 3 months, the grafts were completely lined with ciliated columnar epithelium in 7 (Fig. 12A,B). In 3, both cuboidal and ciliated columnar epithelium were present. In the pig that died, only cuboidal epithelium lined the luminal surface of the graft. The area of infolding which was responsible for the original tracheal stenosis was represented by a "knot" of cartilage at the posterior junction of the graft and tracheal cartilage (Fig. 11). The trachea above the graft demonstrated normal respiratory epithelium.

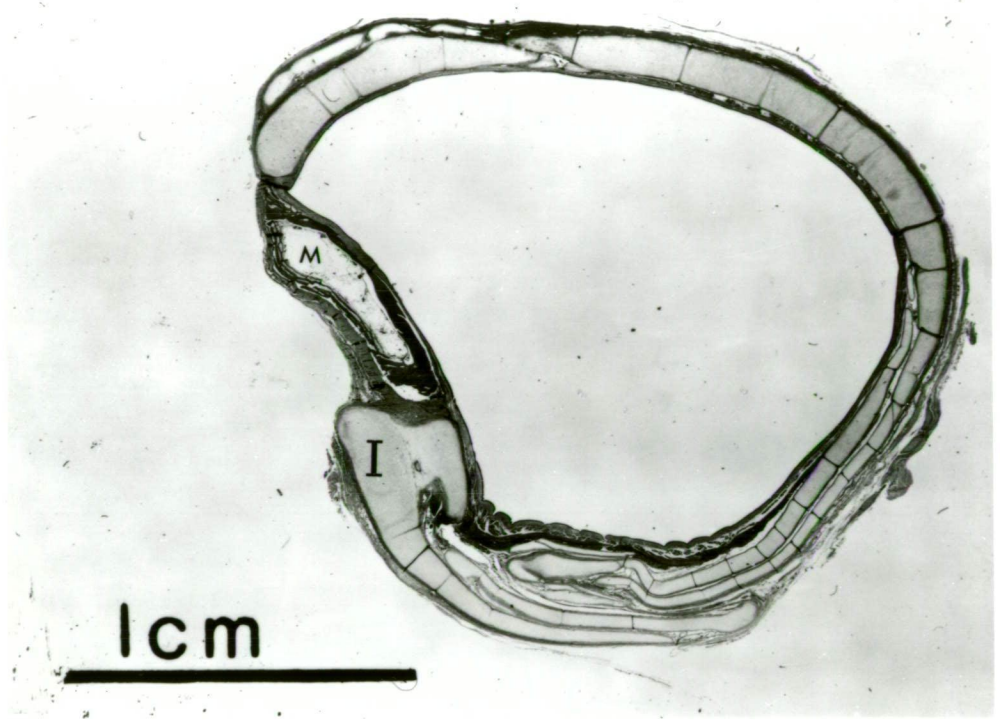


Fig 11. Ossified graft with central marrow space (M)
Area of previous infolding (I).

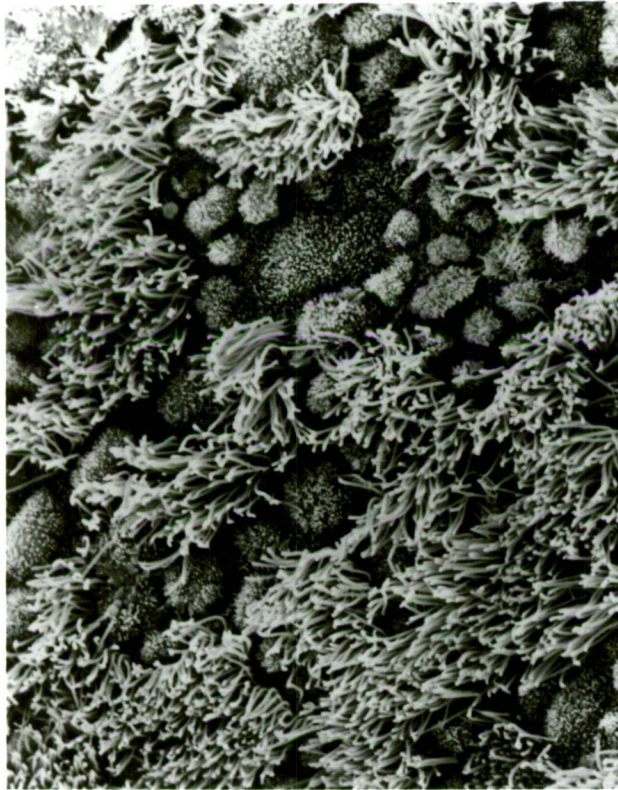


Fig 12A. Epithelium on luminal surface
of the graft 22 days after
repair (x4,800)

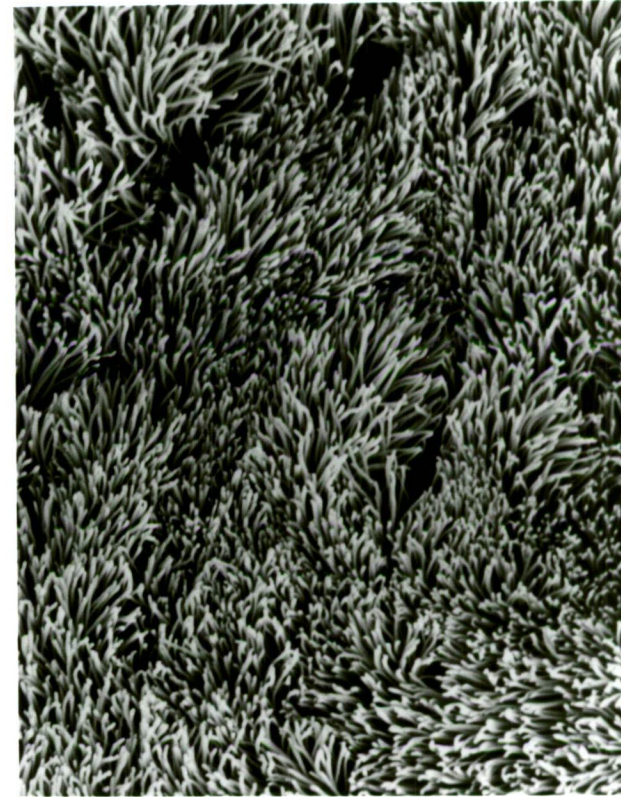


Fig 12B. Ciliated epithelium on luminal
surface of the graft 3 months after
repair (x4,800)

DISCUSSION

Congenital tracheal stenosis is a lethal condition characterized by an absence of the membranous portion of the trachea with fusion of each tracheal cartilage posteriorly throughout the length of the stenotic segment (Cantrell, 1964). In the initial experiments on piglets, a window defect was created in the thoracic trachea similar to that which would be produced when congenital tracheal stenosis in children was treated by longitudinal incision. These defects were successfully repaired with free tibial periosteal grafts. However, these repairs were performed on normal tracheas. In the second phase of this study, a model of tracheal stenosis was created in a short and predictable period of time which had features similar to congenital tracheal stenosis seen in children: the tracheal rings were permanently fused posteriorly by dense collagen at the point of infolding, obliterating the membranous portion; an inflammatory reaction was present around the stenotic segment of the trachea and signs of airway obstruction occurred which were irreversible and resulted in the animal's death.

The pig trachea resembles the human trachea anatomically and functionally (Hare, 1975; Munoz, 1984). Posteriorly, the tracheal muscle is attached to the slightly overlapping cartilagenous rings which normally separate as the trachea expands with inspiration and is compressed during "grunting".

The Silastic cuff appears to induce stenosis by limiting tracheal growth and altering the normal dynamic activity of the trachea so that expanding and compressing forces are repeatedly applied to the immobile tracheal rings. The weak membranous segment infolds with the resultant stenosis and intraluminal obstruction. Tibial periosteum used to repair the tracheal defect is readily available, tough, pliable, and can be sutured to the margins of the tracheal defect to create an airtight seal. There was no obvious abnormality of growth in relation to the tibia from which the periosteal graft was taken. Radiographs and histopathological studies in one pig confirmed this impression.

All the periosteal grafts were orientated so that the osteogenic layer faced into the tracheal lumen as there was some evidence that this would improve ossification of the graft (Kufas, 1981). Ossification of the graft occurred by the third post-operative week. In two pigs that developed stenosis following repair, neither had evidence of graft ossification. Although this does not represent a statistically significant number, it suggests that ossification of the graft may be an important factor in preventing stenosis.

In the initial studies, omental pedicle graft was not essential for the vascularization of the periosteal graft used to repair defects in the normal trachea. In the tracheal stenosis repair

model, complete ossification of the FPG occurred when OPG was applied to it whereas ossification was incomplete or absent in five of the ten animals in which OPG was not used. However, these differences were not statistically significant.

Areas of mature ciliated respiratory epithelium were seen over the graft surface as early as the 22nd post-operative day, further maturation and proliferation occurred so that by three months, the majority of the grafts were completely covered with mature respiratory epithelium. Ohlsen and Norden (1976) used perichondrial grafts from rib cartilage for cervical tracheal reconstruction in four dogs. The perichondrial grafts were placed on two fascial flaps raised from adjacent muscle, rotated into the defect and sutured for complete coverage. They also demonstrated that the regenerated mucosa does exhibit normal function by passing mucus rapidly across the reconstructed area, using cardio-green as an indicator.

An increase in mean cross-sectional area was observed to a significantly greater degree at the level of the repaired segment of trachea over the 3-month period. This was due to tracheal cartilage growth, possibly induced by the periosteal graft. Evidence for this hypothesis is provided by the phenomenon of epiphyseal differentiation of the cartilagenous elements at the sites where the

cartilage and periosteum joined. This suggests that if the complete rings in congenital tracheal stenosis in children were incised longitudinally and a periosteal graft inserted, growth of the tracheal rings may be induced by the graft. None of the repaired tracheas demonstrated undue rigidity as a result of the ossified graft. The cartilagenous rings in the repaired segment grew and provided sufficient flexibility for normal movement and function.

This new model of tracheal stenosis in piglets could readily be used to assess the suitability of other graft tissues for tracheal reconstruction. The stenosis occurs in a short period of time and has similarities to congenital tracheal stenosis. The successful repair by longitudinal incision, temporary internal splinting and insertion of a free periosteal graft into the defect may be a suitable option for correction of congenital tracheal stenosis in children.

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